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TITLE: Fish Oil Supplementation and Fatty Acid Synthase Expression in the
Prostate: A Randomized Controlled Trial

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14. ABSTRACT: One in seven men over the age of 60 will be diagnosed with prostate cancer. Elucidation of early cellular changes that may predict progression to prostate cancer and the identification of factors that may inhibit or reverse these cellular changes would be of great clinical significance. Alteration of the fatty acid synthase (FAS) pathway is an early cellular change that has recently come under investigation. Overexpression of the lipogenic enzyme FAS has been noted in several tumor and pre-cancerous tissue types, including prostatic intraepithelial neoplasia (PIN) and prostate cancer and has been suggested as an independent predictor of disease stage. Additionally, inhibition of FAS has been demonstrated to induce apoptosis and reduce cell proliferation in cancer cells. Fatty acid synthase expression in cancer and normal cells is regulated by the transcription factor sterol regulatory element binding protein 1c (SREBP-1). The up-regulation of SREBP-1 in tumor cells results in increased FAS expression and fatty acid synthesis. Research in normal cells has demonstrated that dietary supplementation with polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids, inhibits SREBP-1 activation, resulting in a decreased transcription of FAS.				
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INTRODUCTION

We are conducting a double-blind, placebo-controlled, randomized intervention study to evaluate the effects of Fish Oil (FO) supplementation on markers of lipid metabolism in prostate tissue samples. The primary endpoints of this trial are fatty acid synthase expression, caveolin-1 expression, changes in lipid raft fractions in the plasma membrane and cell proliferation (Ki-67 expression) in benign, pre-neoplastic and neoplastic prostate tissue. The secondary endpoints include measuring the expression of SREBP-1, a transcription factor for fatty acid synthase, cell death (apoptotic fraction using TUNEL), red blood cell (RBC) fatty acid concentration and change in PSA. Subjects are men from the Portland VA Medical Center (PVAMC), the Oregon Health and Science University (OHSU) and Kaiser Permanente Northwest (KPNW) urology clinics who are scheduled for a repeat biopsy. These men will have had an initial negative biopsy yet are still considered at high risk due to continued elevated prostatic specific antigen (PSA >4µg/dl), are positive for PIN, have suspicious findings by DRE or TRUS, or other clinical finding. Approximately 80 men total will be recruited and randomized to receive three months of either fish oil capsules (treatment 1) or olive oil (placebo) capsules (treatment 2). Potential confounding variables are assessed through completion of a comprehensive diet history questionnaire and risk factor questionnaire, assessment of pre and post-treatment PSA and surveillance of medication and supplement use. Compliance will be assessed using pill count and evaluation of RBC fatty acid concentrations. While this study population is limited to men at high risk of disease, the results may be more broadly generalizable to any man considered at risk of prostate cancer due to standard clinical indicators such as a PSA >4µg/ml.

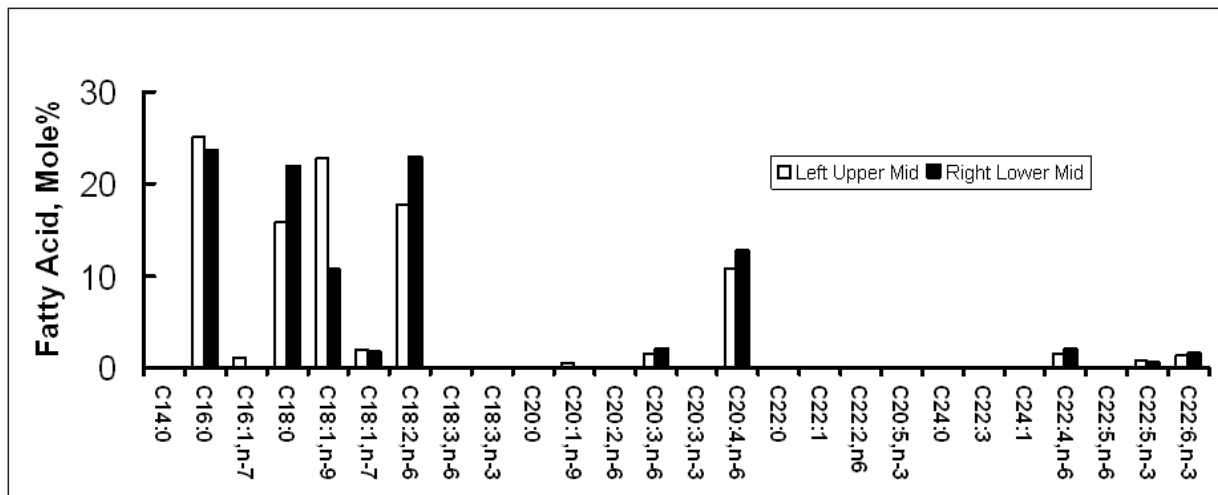
FISH OIL TRIAL RECRUITMENT: SEPTEMBER 2006–MARCH 2010	
Fish Oil Participant Status	
Total subjects referred: 244	N (% of all eligible)
Refusal for any reason:	136
Ineligible	33
Total subjects enrolled :	75
Completed study:	69 (92%)
Active:	2 (3%)
Withdrawn:	4 (5%)
Identified potential subjects awaiting eligibility determination	2
Total subjects enrolled through Fish Oil only trial	30
Total subjects enrolled through Fish Oil and Green Tea	45

BODY

Specific Aims: The aims as presented in the last annual report have not changed.

Studies and Results: During this year's budget period, we have continued our recruitment efforts and are now very near to reaching our proposed 80 subjects. Since February 2009, we have successfully enrolled 19 men into the trial. Two additional men have been referred and will be contacted in the next week to confirm interest and eligibility. These activities are directly related to Tasks 1 and 2 in our Scope of Work (Appendix 1).

In addition, a primary activity over the past year has been to conduct the first batch of laboratory analyses on samples from 50 subjects. Pre and post-intervention biopsy specimens (paraffin embedded and frozen research only biopsy cores) have been obtained from the first OHSU, VA and Kaiser subjects. Immunohistochemical analyses have been conducted at OHSU for fatty acid synthase, sterol regulatory element binding protein (SREBP-1), cell proliferation (Ki-67) and apoptosis. Conditions and procedures for analyses of fatty acid levels in the frozen prostate biopsy core have also been worked out and the first 16 specimens have been shipped to our colleague at OSU for analyses. As shown in Figure 1 below shows Dr. Jump has been able to successfully extract and quantify fatty acids from frozen biopsy cores (graph shows two cores from the same individual).



Dr. Jump will also begin plasma fatty acid analyses in Spring 2010 and it will then be possible to correlate plasma and tissue fatty acid concentrations as well as the association between plasma and tissue fatty acids to tissue FAS and SREBP-1 expression. A final no cost extension will allow for completion of these analyses and importantly will allow for us to obtain the pre and post-intervention biopsy cores for the 25 last subjects recruited through Kaiser Permanente Northwest.

HUMAN SUBJECTS REVIEW: Oversight for our protocol was transferred to USAMRMC HRPO on 1 September 2006; all minor modifications were reported to HRPO at the time of Continuing Review for all three sites. We utilize the HRPO Continuing Review Checklist annually and write corresponding explanation memos for these submissions to HRPO. Summary of local human subjects review follows:

For log number A-12538.a (PVAMC), the DOD HRPO received PVAMC's last Continuing Review (PVAMC approval in January 2010) in March 2010. For log number A-12538.b (OHSU), the DOD HRPO received OHSU's continuing review submission (OHSU approval: January 2010) in February 2010. For log number A-12538.c (KPNW), the DOD HRPO received KPNW'S continuing review submission (KPNW approval: June 2009) in July 2009. The trial underwent an annual Data Safety Monitoring Committee Audit by the OHSU Knight Cancer Institute and was found to be fully compliant. See attached Audit report (Appendix 2).

STUDY COORDINATION: Ms. Courtney Maxcy has taken on the role of primary staff responsible for patient contact and recruitment procedures as well as on-going contact with collaborating clinicians. As described previously, Ms. Maxcy has maintained the

pro-active and aggressive recruitment methods with our collaborating clinicians since taking over study responsibility. She visits the two Kaiser clinics regularly to maintain relationships with the Kaiser clinicians. The new OHSU urologist has been added to our protocol and we have begun to again recruit subjects through the OHSU Urology clinic. Ms. Maxcy continues to work closely with the PVAMC clinician and his nurse to increase recruitment. Ms. Farris retains primary responsibility for human subjects' paper work, continuing review documents and maintains annual contact with Johanna Kidwell (CDMRC). All of Dr. Shannon's team – numbering four – are cross-trained and can assist with subject visits, follow-up and biopsy core processing, as necessary.

PROGRESS TO DATE: We have obtained the first batch of pre and post-intervention prostate biopsy tissue from each hospital's pathology department for data analysis. We have successfully worked with staff at the Kaiser Permanente NW pathology archives to ascertain tissue and will continue to follow this protocol to ascertain the final batch of tissue specimens. The OHSU pathology core and Dr. Christopher Corless, director, have conducted secondary reading of H&E slides for re-cut, stained and conducted immunohistochemistry analyses for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1). Procedures and methods for extraction and quantification of fatty acids in frozen biopsy tissue have been developed and tested, and tissue and blood specimens are being shipped in batches to OSU.

KEY RESEARCH ACCOMPLISHMENTS: Our key accomplishments over the past year have been to continue successful trial recruitment, develop the necessary protocols for laboratory analyses and conduct the first batch of immunohistochemical analyses.

REPORTABLE OUTCOMES: None to date

CONCLUSIONS: We continue to recruit successfully to the trial and have taken major steps toward completing recruitment and initiating laboratory analyses. We have received a no-cost extension to allow for us to obtain final biopsy tissue specimens and complete all laboratory analyses.

C. Revised Statement of Work, version 5

Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate: A Randomized Controlled Trial

Task 1. Finalize clinical protocol and training: Months 1-6 **(Completed)**

- a. Develop tracking system for recording patient recruitment, contact and consent information.
- b. Obtain IRB approval from Portland VA Medical Center (PVAMC), Oregon Health and Sciences University (OHSU) and Kaiser Permanente Northwest (KPNW).
- c. Finalize encapsulation procedure and obtain treatment and placebo capsules.
- d. Finalize and review clinical protocol with GCRC nursing staff.
- e. Review and optimize blood processing procedures with laboratory staff.
- f. Review procedures for patient contact and recruitment with (PVAMC) Mark Garzotto, MD, Laura Peters, RN and study coordinator, Amy Palma.
- g. Modify tracking system, protocol, and consent form to allow for the collection of an additional prostate biopsy core to be cryopreserved.

Expected Product: Tracking system, IRB approval, IRB approval of amendment.

Task 2. Subject recruitment and data collection Months 1 – 9 (Complete by 5/2009)

- a. With the addition of two study sites (IRB and DOD approved); review procedures for patient contact and recruitment with (OHSU) Mitchell Sokoloff, MD and Mark Johnson, RN; (KPNW) Stephen Lieberman, MD.
- b. Patient Eligibility and Recruitment:
 - 1. Pre-Recruitment Screening**
 - Clinician recommends repeat biopsy of the prostate
 - 2. Inclusion**
 - Age 21 years or older
 - Signed informed consent form
 - 3. Exclusion**
 - Definitive prostate cancer on initial biopsy
 - Significant active medical illness that in the opinion of the clinician would preclude protocol treatment.
 - History of ventricular tachycardia or ventricular fibrillation
 - Patient reported use of fish oil (at greater than 1 gram per day) or green tea supplement within 30 days before Day 1 of study treatment
 - Use of warfarin or need for therapeutic anticoagulation at time of biopsy or at anytime during the course of the trial.
 - Subject reported allergy or sensitivity to fish oil, olive oil or green tea
 - Subject reported history of hemophilia, van Willebrands disease or other bleeding disorder, except when the subject is evaluated by a hematologist who determines that fish oil supplementation is not contraindicated.
 - Total bilirubin greater than institutional upper limit of normal
 - VA subjects may not be a part of another 'flagged' high risk study as noted, in red, on the cover sheet of subjects' VISTA/CPRS electronic medical record.
- c. Initial telephone contact and schedule appointment.
- d. 1st visit – at the OHSU GCRC-- Informed consent process; Initial study procedures:
 - i. Outpatient specimen collection form (including height, weight and blood pressure) and inquire about recent history of concerns that would preclude phlebotomy
 - ii. Blood draw for baseline red blood cell fatty acid assessment (10mL)

- iii. Blood draw for analyses of serum osteocalcin (10mL)
- iv. Blood draw for baseline total bilirubin test (5mL)
- v. Urine collection for measures of bone turnover
- vi. Study Questionnaires (Adverse Event and Diet History Questionnaire (DHQ) and Risk Factor Questionnaire)
- vii. Randomization
- e. Eligibility confirmation -Four week supply of placebo or treatment capsules distributed.
- f. 2nd visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
 - Four week supply of placebo or treatment capsules distributed.
 - Complete side-effects and adverse events reporting form.
- g. 3rd visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
 - Four week supply of placebo or treatment capsules distributed.
 - Complete side-effects and adverse events reporting form.
- h. 5th visit – at PVAMC clinic area E or OHSU or KPNW Urology clinics
 - Return unused capsules.
 - Obtain 20 ml blood specimen
 - Repeat biopsy conducted per standard clinical procedure (this is not a study linked event)
 - Obtain two additional biopsy cores for cryopreservation and analyses of lipid raft fractions. In men with known prostate cancer this core will be taken, if possible, from the quadrant farthest from the known tumor. Fresh tissue collected at surgery by study RA and delivered immediately to the OHSU Pharmacokinetics Core for cryopreservation.

Expected Product: Questionnaire, blood specimen and biopsy (frozen and paraffin embedded) data for **80** patients (original number for which funding was received).

Task 3. Preparation for Immunohistochemistry (IHC) / Data Entry. Months 13-24 (**Complete**)

- a. Optimize IHC for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1)
- b. Optimize protocol for lipid raft extraction from tissue specimens (using stored non-study tissue).
- c. Develop database for tracking specimen receipt and analysis
- d. Begin data entry of questionnaire forms and event reporting forms

Expected Product: High functioning antibodies and procedures for FAS and SREBP-1 IHC, final procedures for lipid extraction and raft associated protein analyses, laboratory database, complete questionnaire data entry.

Task 4. Laboratory / Dietary Analyses.

Months 1-6

- a. Blood specimens **and prostate biopsy cores** shipped to ~~Seattle~~ Oregon State University for plasma and tissue fatty acid analyses.
- b. Initial and repeat biopsy specimens obtained from PVAMC / OHSU / KPNW pathology. **(Complete for first batch of 50 subjects)**
- c. Perform IHC for FAS and SREBP-1 on pre and post intervention tissue specimens. **(Complete for first batch of 50 subjects)**
- d. Perform IHC for Ki-67 and TUNEL assay on post-intervention tissue specimens. **(Complete for first batch of 50 subjects)**
- e. Perform full fatty acid panel analyses on post-intervention frozen tissue using HPLC. **(Methods developed and tested, complete for first 10 subjects)**
- f. Run nutrient analysis program on diet history questionnaire data.
- g. Data cleaning.

Expected Product: Complete data on FAS and SREBP-1 expression in **160** tissue specimens from **80** patients. Complete data on Ki-67 expression and TUNEL for **80** tissue specimens from **80** patients. **Complete data on distribution and type of fatty**

acids in the frozen biopsy specimens. Plasma fatty acid concentrations from **160** blood specimens from **80** patients. Nutrient intake data from 80 patients.

- Task 5.* Final Analyses and Report Writing Months 7-12
- a. Final analysis of data from questionnaires, blood specimens and tissue specimens will be performed
 - b. Prepare final report and initial manuscripts.
- Expected Product:* Completed and submitted final report a minimum of 1 submitted manuscript.